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OIL FROM PLANTS

Melvin Calvin

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OIL FROM PLANTS

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ABSTRACT

As a result of the exhaustion of our supplies of ancient photosynthesis (oil and gas) it is necessary to develop renewable fuels for the future. The most immediate source of renewable fuel is, of course, the annually growing green plants, some of which produce hydrocarbon(s) directly. New plant sources can be selected for this purpose, plants which have high potential for production of chemicals and liquid fuels. Suggestions are made for modification of both the product character and the productivity of the plants. Ultimately, a totally synthetic device will be developed for the conversion of solar quanta into useful chemical form completely independent of the need for arable land.

Presented at BARC Science Seminar, Beltsville Agricultural
Research Center, U.S. Department of Agriculture,
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INTRODUCTION

Growing oil, my subject for this paper, means just that. Up until now, we have been mining oil, the ancient photosynthetic product of several hundred million years ago. The question now most frequently arises about new sources of ancient photosynthetically produced materials, and there is no longer a tenable positive answer to this question. Other alternatives must be found.

An immediate need for alternate energy sources is emphasized by the history of productivity of drilling rates for U.S. domestic petroleum from 1945 to the present time. It has been demonstrated that the amount of oil found per foot of well drilled is falling, from 35 barrels of oil per foot drilled in 1945 to less than half that amount in 1975; the rate of discovery is falling. Also, the energy cost of drilling and extracting oil is rising.¹ The productivity is falling, the cost of obtaining the oil is rising in terms of energy and somewhere near the year 2000 the energy cost of finding and extracting a barrel of oil will exceed the energy content of that barrel of oil. When that occurs, even if there is oil in the ground, the energy cost of extraction will prohibit any further use of the oil as it will cost more, in energy terms, to extract it than it will be worth.

CARBON DIOXIDE PROBLEM

One alternative which has been suggested is to use coal (also a product of ancient photosynthesis), huge supplies of which are available in the United States, the Soviet Union, Europe and China, but this also has some problems. About 20 to 30 years ago we were admonished to restrict the use of coal for environmental reasons, such as the destruction of the land by strip mining, the destruction of the miners by deep mining, the destruction of the people by the effluents of burning coal, the effluents consisting of acid rain and unburned hydrocarbons which are carcinogens. We transformed our coal burning power plants into clean (gas) or low sulfur (oil) burning power plants to eliminate or reduce environmental hazards. As a result of the oil embargo in 1973, there has been a return to the idea of using coal for power plants, especially by indirect combustion (coal conversion to gas or liquid hydrocarbons which would be useful) rather than by older combustion methods. When we suggest that coal in any form can be used as an alternative to burning oil, there will be an environmental constraint to prevent the use of coal power over a very extended period.

The combustion of fossil carbon in any form, but particularly coal, has created a problem, particularly over the last 100 years but in an accelerated form in the last 20 years. This is a result of the combustion of fossil carbon, and it does not matter in which form the fossil carbon is burned. Carbon which has been stored in the ground for several hundred million years and is suddenly used generates excess carbon dioxide. It is not possible to get the heat/energy values from the combustion of fossil carbon (oil or coal) without the production of CO₂. As a result, there has been a 7% increase in the CO₂ levels in the atmosphere over the past 20 years, even just burning mostly oil which is not just carbon but carbon and hydrogen; oil has approximately two

atoms of hydrogen for every atom of carbon, and the heat comes from the burning of hydrogen as well as the burning of carbon. When coal is burned, however, there is less than one atom of hydrogen for one atom of carbon, with the result that roughly twice as much carbon dioxide per million Btus is created than from the burning of oil.

It is possible to observe the annual increase in the CO₂ levels in the atmosphere from the data in Fig. 1, which is taken from a station at the top of Mauna Loa in Hawaii. This site was chosen because of its distance from urban and natural disturbances, with the result that the data obtained is more truly representative of the actual atmospheric situation as it is not contaminated with other pollutants. (There are other stations throughout the world where information about CO₂ levels is obtained. The suggestion was made some years ago that it would be important to establish a similar station in the tropical forests in the Amazon to obtain the vertical CO₂ profile so that the effect of the forest itself on this increase could be observed.) The annual changes (rise in the winter and fall in the summer) of the CO₂ levels in the atmosphere are clearly observable. Notice that the fall in the summer is never as great as the rise in CO₂ concentration in the winter, with the result that the net CO₂ level is rising constantly since 1958 when the station on Mauna Loa was established, from 315 ppm to over 330 ppm. We can actually extrapolate backwards by another type of measurements, the carbon-13 content of tree rings which were laid down in 1860, and from the ¹³C deficiency we can estimate the total carbon dioxide which was in the atmosphere in 1860; it turns out to be about 290 ppm for that time. So, from 1860 to 1980 the rise has been about 15% in CO₂ concentration and of that 15% one-half has been in the period 1958 to 1981. There has been a slight decrease in worldwide carbon-based fuel usage since the oil embargo period, but that is a relatively small perturbation on the overall effect of the rise of the CO₂ concentration.

Why is the CO₂ level important? Carbon dioxide is a peculiar gas. It is transparent to visible light and approximately 99% of the visible light is converted to heat when it strikes the surface of the earth, after passing through the atmospheric CO₂ blanket. This heat is re-irradiated back into space, but the carbon dioxide is opaque to infrared light and absorbs it and re-reflects some of it back down to the earth's surface. The CO₂ blanket thus acts as a one-way valve letting heat into the surface of the earth, not allowing the heat to escape again into space, with a result that the earth's surface temperature rises.^{2,3}

Effect of Rising World Temperature. Let's discuss for a moment what the rising temperature of the earth may be expected to be and what the possible economic and social costs might be as a result. Estimates have been made by various means and the data obtained show the rising temperatures as a result of synthetic fuel use which might be as great as 40 from what it is today, a very large change in the global average temperature. Even if we now use nonfossil replacement fuels, with fast growth, we still have the inertia of the CO₂ rise which has already begun and which will continue, as will the temperature rise. Synfuels with no growth and non-fossil replacement fuels with no growth still show an increase in the pro-

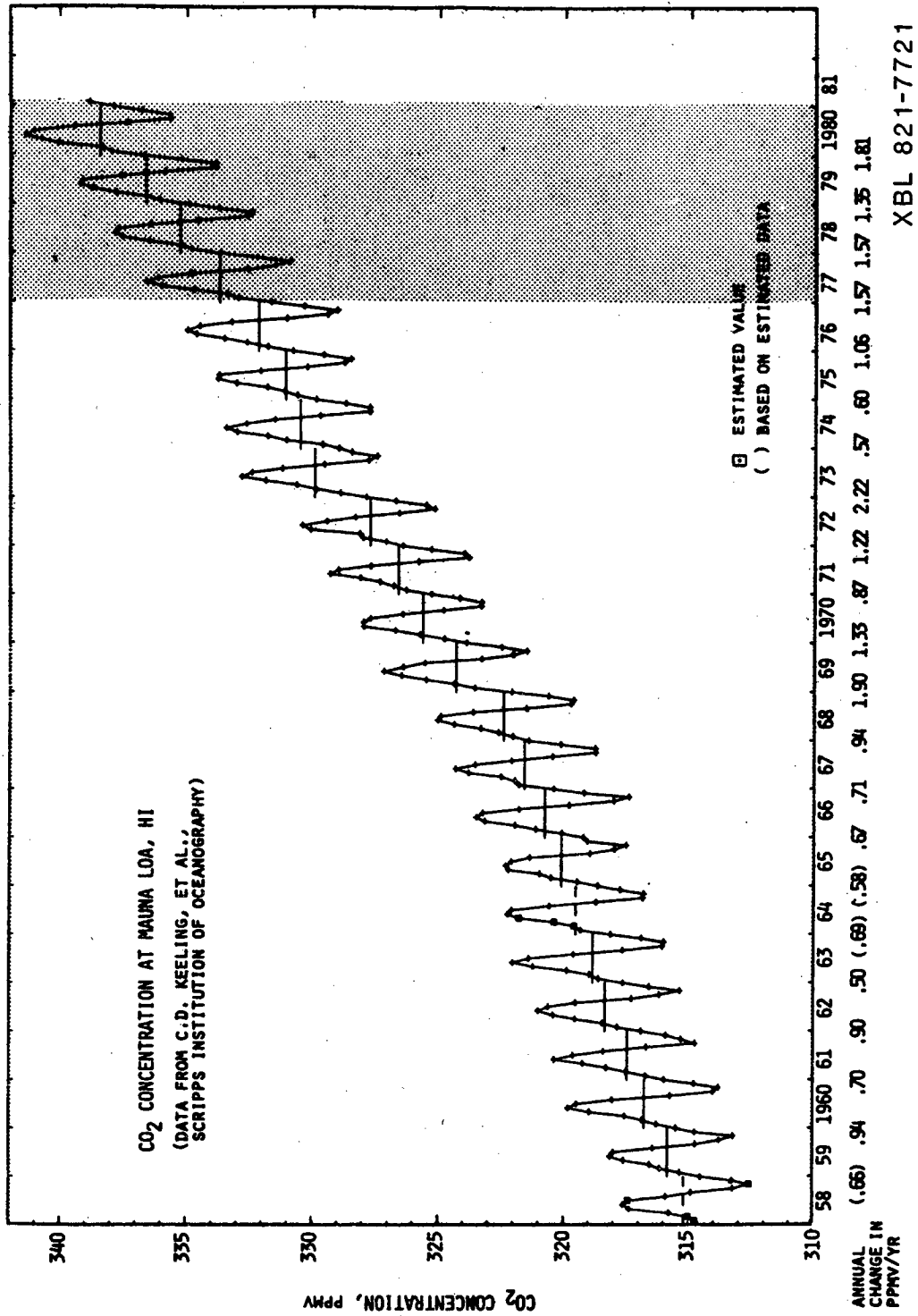


Fig. 1

XBL 821-7721

jections for global temperature. There is no way to stop the temperature rise which has already begun, and it will take 50 to 100 years before the temperature rise levels off or even begins to fall again. We can be sure that if such temperature rises take place there will be profound effects on agriculture on a global scale, on human distribution, and on human societies which will have to adjust to a relatively very rapid change.

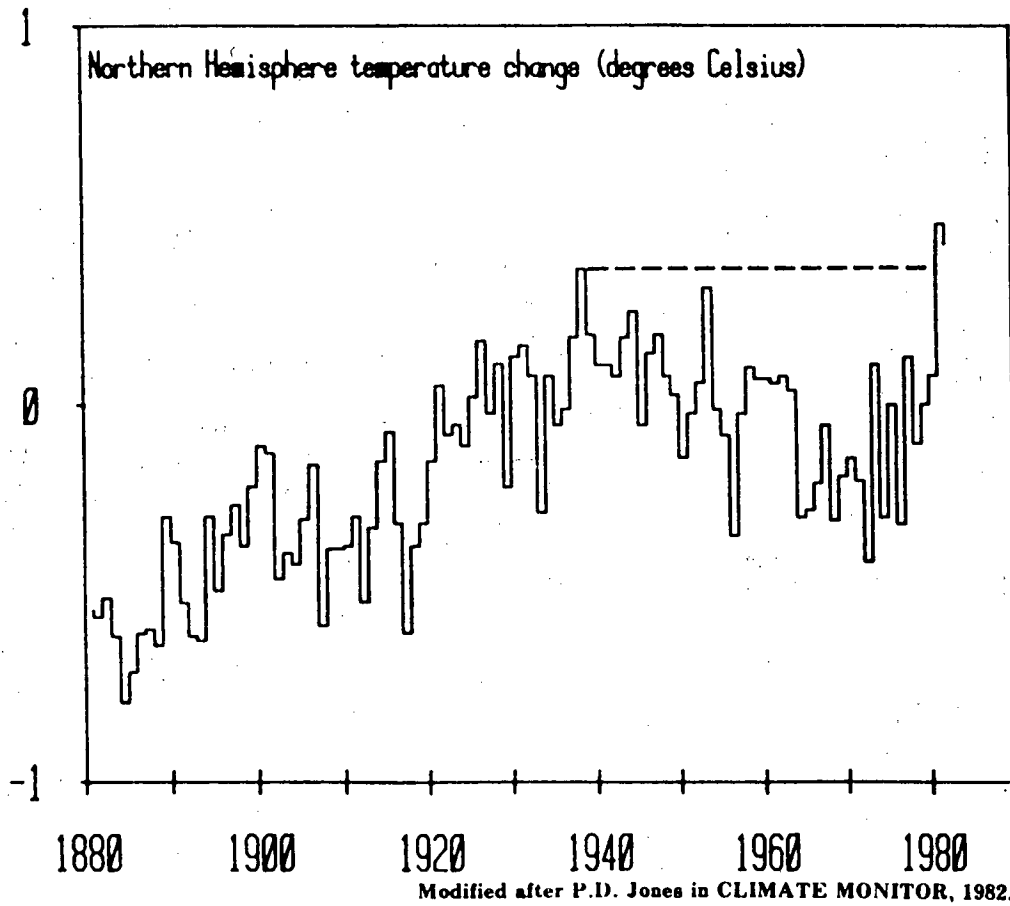
Several years ago the National Academy of Sciences organized a study on the effects of the carbon dioxide concentration increase in the atmosphere and in 1982 they updated their findings.² The 1982 report indicated that the increase in temperature of 3° postulated in 1979, which was predicted to accompany a doubling of atmospheric CO₂ concentration, needs "no substantial revision" at this time. The potential of increasing concentrations of atmospheric CO₂ to produce climatic effects is substantial, and the scientific uncertainties which surround the nature and extent of these factors require extensive worldwide monitoring of temperature, ocean cycling and other factors.

Is it possible to detect any consequence of the 15% rise in the CO₂ level which has occurred in the last 100 years? Can we extract this rise from the "noise" of the annual fluctuations in time and in space of the temperature and weather in general? A number of efforts have been made to find "early warning signals" large enough to be believable but not so serious as to cause agricultural, economic and social damage. If we wait until the evidence is unambiguously clear, until the temperature actually rises unambiguously, it will be too late to alter our living patterns to depart from the inertial curve. We need some "early warning signals" to inform us about whether the temperature rise is happening and the order of magnitude of its occurrence.^{4,5} I have collected three or four different "signals" and they are independent observations, but when taken together I believe you will agree that they are unambiguous evidence that the heating up effect is already beginning to show. The simplest is the temperature history of some locality, and the temperature change data for the Northern Hemisphere from 1880 to 1981 are shown in Fig. 2 indicating that 1981 was a peak year for warm weather; however, there are tremendous fluctuations in this data. The rise in temperature from 1880 to 1980 is substantial, even allowing for the large "noise" level from year to year. This is not too good an early warning "signal" because the annual fluctuations are so great it is hard to distinguish a trend. Evidence has also been accumulating that the ocean surface temperature is also increasing as a result of increased atmospheric CO₂ concentration, and an extrapolation has been made as to the time dependent response of the ocean temperature to a possible instantaneous doubling of CO₂ concentration (Fig. 3).²

A more sophisticated piece of evidence concerns the polar ice cap. If the global temperature is indeed rising, one would expect that the polar ice caps would show some consequence of that increase as they are an accumulation of many years. The accumulation of ice on the polar ice caps is an integrated effect unlike the fluctuations in annual temperature. (Temperature measurements are point measurements whereas the amount of

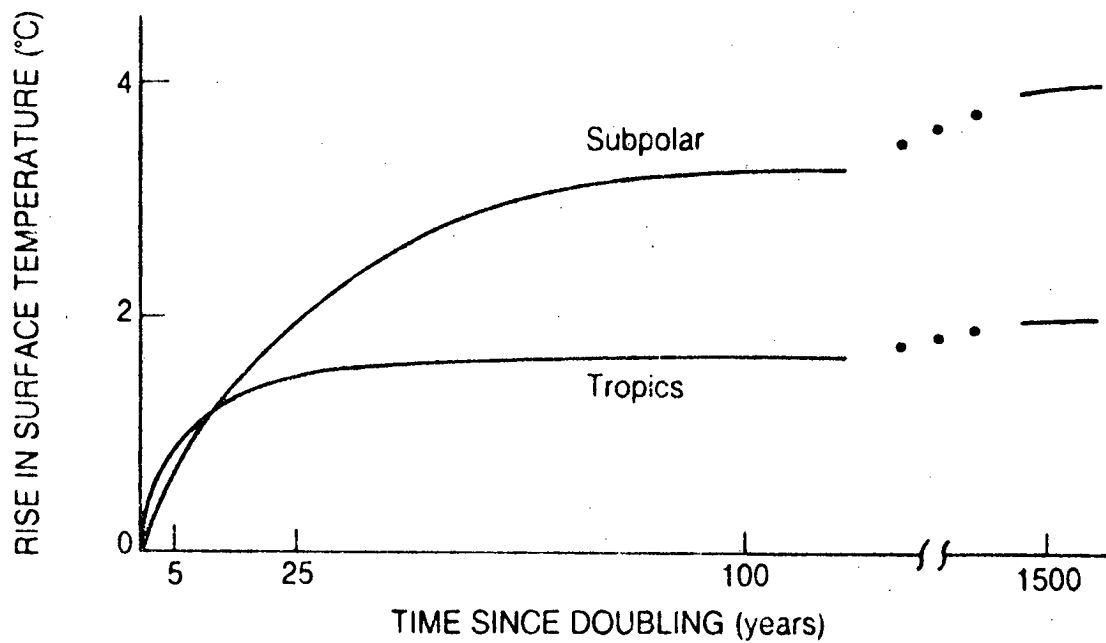
Fig. 2

1981 sets modern heat record



XBL 828-10889

Fig. 3



XBL 8211-3470

Time-Dependent Response of
Ocean Surface Temperature to
Doubling of Atmospheric CO₂
(National Academy of Sciences,
1982)

polar ice is an integrated measurement of the amount of heat coming through and leaving the earth's surface and it smooths out the "noise" level). Last year there was a satellite photograph of the South Polar Ice Cap, and that photograph, together with surface measurements by the U.S. and Soviet navies has provided evidence that the South Polar Ice Cap has decreased in the last 100 years. This data indicates that the South Polar Ice Cap has been diminishing, and in the last 7 years it has fallen from about 12 to 13 million square kilometers to near 10 million square kilometers, in other words, 2 million square kilometers of ice have disappeared (melted) from the South Polar Ice Cap. From temperature distribution data on the ice cap it is possible to state that the thickness of the remaining ice cap has also decreased.⁶ One can calculate how much ice has melted and, therefore, how much "new" water has come into the oceans. We can then deduce that the sea level should have risen and more rapidly in the last 20 years than before. The global mean sea level trend based on tide gauge measurements indicates that from 1800 to 1940 the rise was about 1 mm/year and from 1940 to 1980 the rise has been 2 to 3 mm/year. That, I think, is unambiguous evidence, together with the satellite data and the temperature information for the Northern Hemisphere, that the heating effect (greenhouse effect) of the CO₂ is indeed occurring and will have its consequence on agricultural patterns throughout the globe in various ways. Unfortunately, we do not as yet have good enough meteorological models to predict microclimatic changes,⁷ i.e., to predict where the heat will be greatest, where the rainfall will be increased/decreased, where the agricultural patterns of the globe will change during the relatively brief period of time (20 to 30 years) which may be available.

To give some clue as to what the surface change in the sea level might become we can examine a period about 50,000 years ago when the temperature was higher and there was less polar ice. We find evidence, at least on the east coast of the United States, which is unambiguous. It has been determined that most of Florida was totally under way, or marshland, and a good bit of the east coast was flooded or marshy from New Jersey south to Florida. This is what happened about 50,000 years ago when the Antarctic ice was less than it is today. We currently are going in that direction once more, with the decreasing South Polar Ice Cap, increasing global temperatures and other results of increased atmospheric CO₂ concentration.

What is the alternative? We can't burn coal very long. If we do, we are headed toward major temperature changes. Therefore, the coal burning alternative is only of limited use, for a short time. I believe that coal should not be used at all for environmental reasons, but the temperature change effects alone can and should limit the use of coal.

PLANTS AS ENERGY SOURCES

We are left with only one alternative, and that is some way of harvesting the sun on an annual basis and not simply using the sun's harvest which has been stored in the ground for several hundred million years. As a first alternative, we must learn to harvest the sun annually, which means growing plants which will produce the kinds of materials we need.⁸ We already



Fig. 4 Energy Cane, Puerto Rico. (Photo: Gene Elle Calvin)

CBB 822-1649

are doing that today on a good scale in the form of forestry activities, but these are minor compared to what must be done to meet the fuel and material needs of the next generation. The fact that we can use green plants as a source of almost all the necessary fuel and materials is established through the photosynthetic carbon cycle itself.

Sugar Cane-Puerto Rico. In some countries, energy agricultural practices have already begun. About 10 years ago, just before the oil embargo, the Puerto Rican government constructed a very large power plant on the south coast, intended to be fueled by oil. That became impractical, but Puerto Rico has an alternative to oil. It is possible to grow sugar cane there on a large scale, and research activities have led the Puerto Rican government to encourage the planting of a special type of sugar cane, called "energy" cane. Normally sugar cane is bred to reduce the fiber content and raise the sugar content so the processing of the cane for sugar can be economic. However, in Puerto Rico they realized that the fiber from the cane could be used to run the power plant. They therefore returned to an examination of some old clones which were poor in sugar and rich in fiber. These were planted experimentally a few years ago and the Puerto Ricans have now developed a type of "energy" cane (Fig. 4).⁹ The "energy" cane contains only about 8 to 9% sugar. Actually, the sugar produced per acre from the energy cane is about the same as in the standard (Gran cultura) cane which is about 14 to 15%, but it contains about three times as much fiber (bagasse) which is the important part of the cane for fueling the power plants. By utilizing energy cane Puerto Rico now has both sugar and fiber (fuel) which can be fed into the power plants.

Sugar Cane-Brazil. When we first began looking for energy alternatives back in 1974, I knew there existed plants that were commercially producing hydrocarbons on a large scale, namely, the Hevea rubber trees mostly in Malaysia and Indonesia, and that these Hevea rubber trees were native to the Amazonian forest in Brazil and had been transferred from Brazil through Kew Gardens in London to the southeast Asian plantations where they grow today. I thought if Hevea is native to Brazil, belonging to the family Euphorbiaceae, and making hydrocarbons, might there not be other members of that family, or even of that genus, which would produce hydrocarbons of lower molecular weight which might be useful as crude oil.

It was in Brazil on a trip in 1974 that we learned about the sugar cane industry and how it can be a self-contained, energy-efficient process. Brazil grows more sugar cane than any country in the world, and in 1974 they produced 10 million tons of raw sugar and 400 million liters of fermentation alcohol from the molasses of that sugar. As we traveled around Brazil looking for other species of Euphorbiaceae, we saw mostly sugar cane, and we visited mill after mill, finding that the Brazilians were harvesting the cane under conditions of maximum sucrose production.

If the Brazilians wanted to use alcohol as a possible source of liquid fuels and materials, the cane should be harvested for maximum fermentables rather than maximum sucrose. It was easy to determine that if the Brazilians

waited for the sugar cane to ripen (for the glucose and fructose phosphate to dimerize) some of the fermentables would be lost because the dimerization is an energy consuming process. However, if they harvested the sugar cane at the point of maximum fermentables, that is, a little earlier, they should have a higher alcohol yield. Indeed, the Brazilians now harvest a few weeks earlier and get 10 to 15% more alcohol/hectare than they do when the sugar cane is grown for sucrose production only.

In 1975 the Brazilian government made a decision to go into the alcohol-for-fuel program, what they call the Pro-Alcohol program, and this encouraged the growing of cane for fermentables, which meant modifying the growing procedures, modifying the mill equipment to use the sugar juice directly, etc. The sugar mills themselves became self-supporting in energy in the sense that they had enough bagasse from the cane to generate the steam to run the crushers, to run the fermentation process, to distill the alcohol and fuel the trucks that collected the cane. The products from these mills were excess electricity and alcohol.

The Brazilians are now reaching the stage where they can use the excess electricity from the sugar cane operation to produce nitrogen fertilizer which is required for the soil for cane plantations. The potassium and phosphorus in the ash from the furnaces is available, but the nitrogen is gone from the soil so it is necessary to find a way to replenish it. The excess electricity from burning the bagasse can be used to electrolyze water, to make hydrogen and build small ammonia generators. The sugar plantations of Brazil therefore will be the first completely self-contained "energy plantations", which can not only produce sugar but also produce liquid fuels (alcohol) and petrochemicals from the alcohol which comes from the fermentation process.

HYDROCARBON-PRODUCING PLANTS

Our trips to Brazil and elsewhere (Malaysia, Australia, Spain) were taken for the purpose of finding plants which could produce hydrocarbons of low molecular weight as alternative fuel and materials sources.⁸ We continued to look for species in the family Euphorbiaceae, and we found a very large number of the genus Euphorbia, having roughly 2000 species, all of which produce a latex (similar to Hevea latex) containing one-third hydrocarbon, one-fifth protein and the balance, water. Not only are there 2000 different species of Euphorbias growing all over the world and under all kinds of conditions and growth habits (tiny plants to large trees), but these growth habits and circumstances can be selected for the kind of land and climate which is available.¹⁰

One of the best candidate for hydrocarbon production is Euphorbia lathyris (commonly called the "gopher plant"), and plantations of this species have developed in various parts of the world, specifically in the southwest of the United States,¹¹ California^{8d} and Spain (Fig. 5). There are currently plantations of E. lathyris in other Mediterranean countries, Africa, the Canary Islands and Australia.¹² The reason for our choice of E. lathyris was its universal distribution throughout the United States, growing as far north as Montana and in the Southwest and



Fig. 5 Euphorbia lathyris, Spain

CBB 821-533

even on the east coast. It requires only 12 to 15 inches of water annually and can grow in land which is not suitable for food production. The reason that E. lathyris might be one of the best candidates for an alternate energy crop comes from several factors. First, it is possible to grow this plant using irrigation on semiarid land not suitable for food or fiber production. Second, the plant grows in a 5 to 7 month period to size for harvest. Third, the extraction process is standard for the chemical industry. Also, in addition to the oil, the plant contains a substantial quantity of sugars, fermentable to alcohol. It seems that a yield of 6 to 10 barrels of oil/acre/year is achievable using wild seed. Agronomic and genetic improvements in the plant, of course, would increase that yield.

Because Euphorbia lathyris has been studied more than any other candidate for hydrocarbon production, data is available for many of the factors which are required for a crop of this type, such as genetic variability, irrigation requirements, process development and analyses of the final products.^{10,18} There is no question that in spite of its widespread distribution E. lathyris may not be the best candidate for arid lands as the water requirement is too high and the resulting products not economically competitive with crude oil. Efforts should be made to learn whether or not specialty chemicals from E. lathyris could be used for feedstock development in addition to those fractions which are similar to crude oil, thereby making growing E. lathyris more economically competitive and useful.¹⁸

Another species, Euphorbia tirucalli, a perennial which is common in Brazil, Africa, Israel and which also grows prolifically in the southwest of the United States, has been cultivated for its oil producing quality but it requires more water than E. lathyris. At the present time, Euphorbia tirucalli plantations are being developed in Okinawa and Thailand (Fig. 6), the latter effort actually under the direction of a Japanese chemical company.

There is another plant family, the Asclepiadaceae (milkweeds), which is widely distributed throughout the world. We have seen great areas of some of these plants (Calotropis procera) in Puerto Rico, and they are also being developed in the United States by a private firm, Native Plants, Inc. as a possible candidate for liquid fuel production.¹³ Just recently I have learned from Australia of the existence of plantations of Calotropis procera (Fig. 7) there as well. Several thousand hectares in Australia have been planted with this species, and the effort is being sponsored by the Australian government to determine whether or not this plant would be a suitable candidate for fuel and materials production.¹⁴ The chemistry of Calotropis is similar to that of E. lathyris, and the extraction process that has been developed for Euphorbias and the various transformations can be used with the milkweeds.

The processing sequence to recover terpenoids and sugars from E. lathyris (Fig. 8) is almost the same for E. tirucalli and Calotropis procera.¹⁵⁻¹⁷ This extraction procedure was worked out in the laboratory and has not been used on a pilot plant scale. The process sheet is calculated for 1000 dry tons/day of E. lathyris material which would yield 80 tons of crude



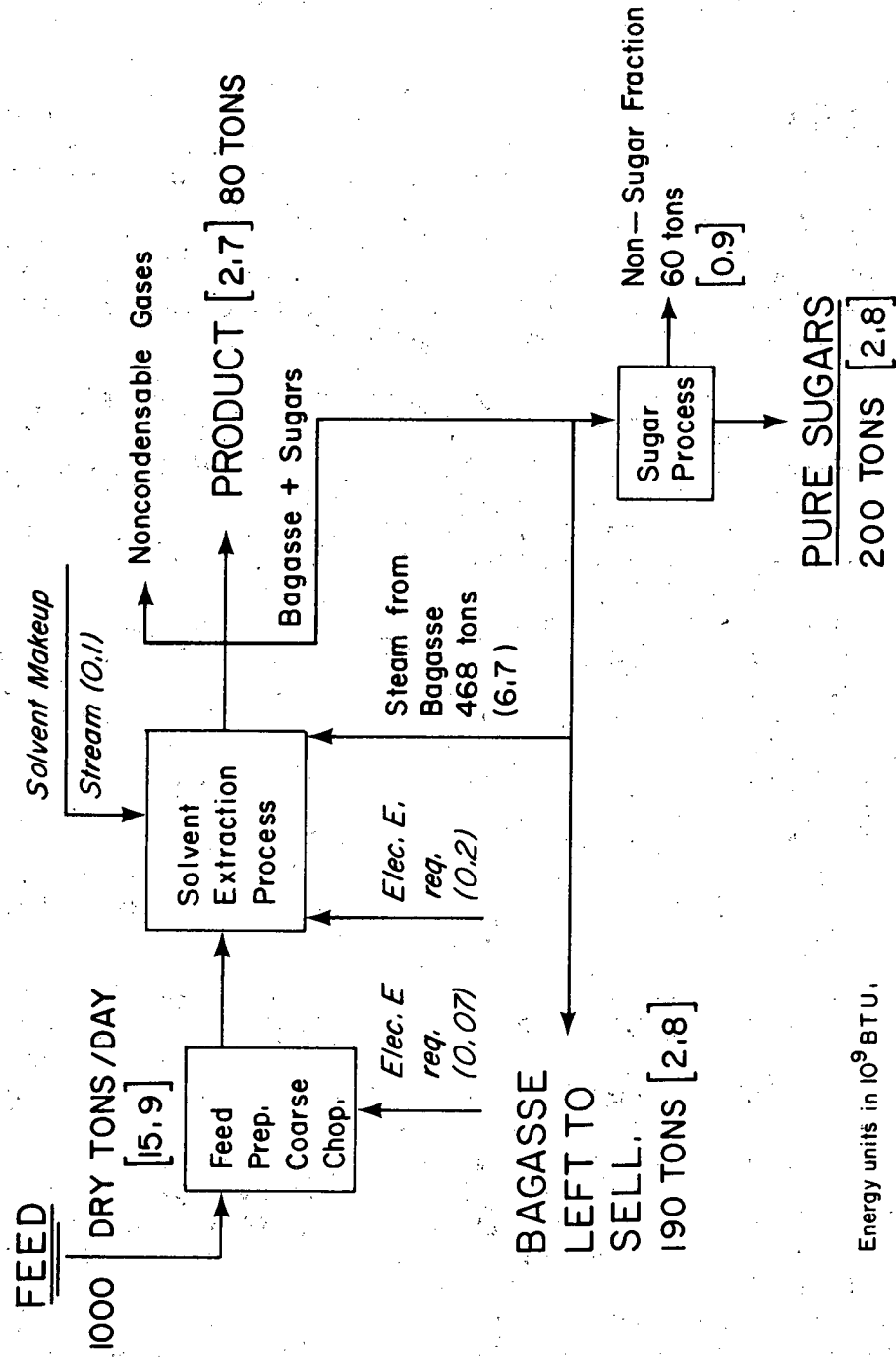
Fig. 6 Euphorbia tirucalli, Thailand. (Yuko Chem. Co., Osaka, Japan)

CBB 820-10452



Fig. 7 Calotropis procera, Australia (Lyll Williams) CBB 829-7854

Conceptual Processing Sequence to Recover Terpenoids and Sugars from Euphorbia lathyrus



XBL 807-4263A

Fig. 3

oil and 200 tons of fermentable sugars which could produce 100 tons of alcohol. About 500 tons of bagasse is used to run the process, with a resulting 200 tons of bagasse which could be used to distill the alcohol. The fermentation alcohol which is a byproduct of processing the dried material from E. lathyris is, of course, a starting point of an entire petrochemical industry.

The whole process is self-contained, as in the sugar cane situation, but the economics of processing E. lathyris and the products recovered from that effort are more conjectural. Some time ago (1980) I made calculations for a small miniplant, and the price that we would have to receive for the oil and alcohol to make the process economically feasible would be \$40/barrel for the oil and \$50/barrel for the alcohol. This type of return would make the process economic, including capital costs for construction of the extraction plant and payments to the farmer for growing the E. lathyris which would be approximately \$500 to \$300/acre.

Studies of E. lathyris have shown that while it is possible to crack the material from this plant, much as crude oil, it might be more economic to try and determine whether this species and its products could contain material more useful for chemical feedstock production, i.e., more specialty chemicals which would not compete with the cheaper crude oil and which would have a more unique situation in world markets.¹⁸

The crude oil from the Euphorbia lathyris has been converted, using special zeolite catalysts developed by the Mobil Corporation, to the usual suite of products which are similar to those from the standard catalytic cracking of petroleum.¹⁸ These include olefins, paraffin, aromatics and nonaromatics. This information confirms the desirability of the products of Euphorbia lathyris as possible raw materials to substitute for crude oil. Since these results were obtained, as a result of additional data on processing and cracking the material from E. lathyris, it now appears that a price of \$100/barrel for the oil would be more realistic. This is still within reason, considering that it is only 2.5 times more than the current (1982) price/barrel for crude from OPEC.

Isoprenoid Formation in "Oil" from Plants and Trees. The chemicals in the plant which constitute the black oil are mostly triterpenes (C₃₀), sterols and sterol esters. It is for this reason that the cracking pattern makes such desirable products. The biosynthetic route to the terpenes in the plant has not been worked out in great detail, but the route in E. lathyris is probably similar to that for rubber biosynthesis, except that the end products from the E. lathyris are lower molecular weight compounds. The biosynthetic route is from sugar via the glycolytic cycle to pyruvate which is then built up to mevalonic acid and goes on to give isopentenylpyrophosphate (IPP). The IPP goes further to polymerize into a variety of isoprenoids. Normally, in the E. lathyris the material goes on through the isoprenoid biosynthetic pathway to squalene (C₃₀) which is then folded up to make the C₃₀ terpenoid alcohols which constitute the greater percentage of the oil. The actual route is: Sugar to pyruvate to acetyl

phosphate to mevalonic acid and on to dimethylallylpyrophosphate (DMAP) and isopentenylpyrophosphate (IPP). The isomeric C_5 compound (DMAP) drops off a pyrophosphate to create a carbonium ion which takes an electron pair from the double bond of IPP to form a five-carbon to five-carbon link, the resulting carbonium ion then drops off the proton to give an identical structure (allylpyrophosphate structure) which can go on, add another C_5 to make a C_{10} compound, then add another five-carbon piece to make a C_{15} compound. This process can continue on to polyisoprenes, essentially to rubber.

However, if the plant contains a cyclase enzyme, the carbonium ion can take an internal double bond and close at a cyclic C_{10} compound instead of adding another five-carbon group. In the case of E. lathyris and E. tirucalli two C_{15} compounds come together to form squalene, a C_{30} open chain with no pyrophosphate which then folds up to form the triterpenes, closed C_{30} compounds, mostly sterols.

Would it be possible to improve the yield of the C_{15} compounds in the plants (which are the desired substances) if the process could be stopped at the C_{15} step? This would mean that no cracking operation would have to be performed on the materials since C_{15} is essentially diesel and the C_{15} cyclic sesquiterpenes can be used directly as diesel fuel without further processing.

Hydrocarbon-Producing Trees. In our search for hydrocarbon-producing plants we encountered a tree in Brazil which can be tapped and which produces sesquiterpenes (diesel oil).^{8f,9,h} This tree belongs to the Leguminosae family and there are several species growing in Brazil. We found one, Copaifera langsdorfii, growing in the Botanical Garden in Rio de Janeiro, but that particular species does not produce as much diesel-like fuel as others. In the Ducke Forest in Manaus we observed the C. multijuga which produces approximately 20 liters of sesquiterpene fuel material per tree in 24 hours every 6 months. The tree is harvested by drilling a hole in the trunk about 3 feet from the ground, the hole is about 2 cm in diameter and goes into the heart wood of the tree. A pipe is inserted in the hole and the oil drains out of the pipe into a bucket (Fig. 9). After the material is "harvested", the hole is plugged with a bung, and 6 months later the tree will produce another 20 liters from the same hole. The oil comes not from the cambium, as does the rubber latex in Hevea, but from the 1 to 2 mm diameter vertical pores in the heart wood.

The material from the C. multijuga is a sesquiterpene which can be put directly into an automobile without any further processing or refining. An experimental plantation of C. multijuga has been started at the Amazon research institute, INPA, in Manaus to show that the sesquiterpenes were "clean" enough to be used directly in automobile engines. They are also studying agronomic practices in connection with this species, with the hope of improving the yield, or perhaps being able to use more than one hole in each tree for diesel fuel production.

The oil from the trees of the Copaifera species, called "copaiba oil", has been analyzed by gas chromatography and three main products are copaene, bergamotene and carophyllene, all C_{30} compounds and all of them are cyclic compounds with C_{15} .²⁰

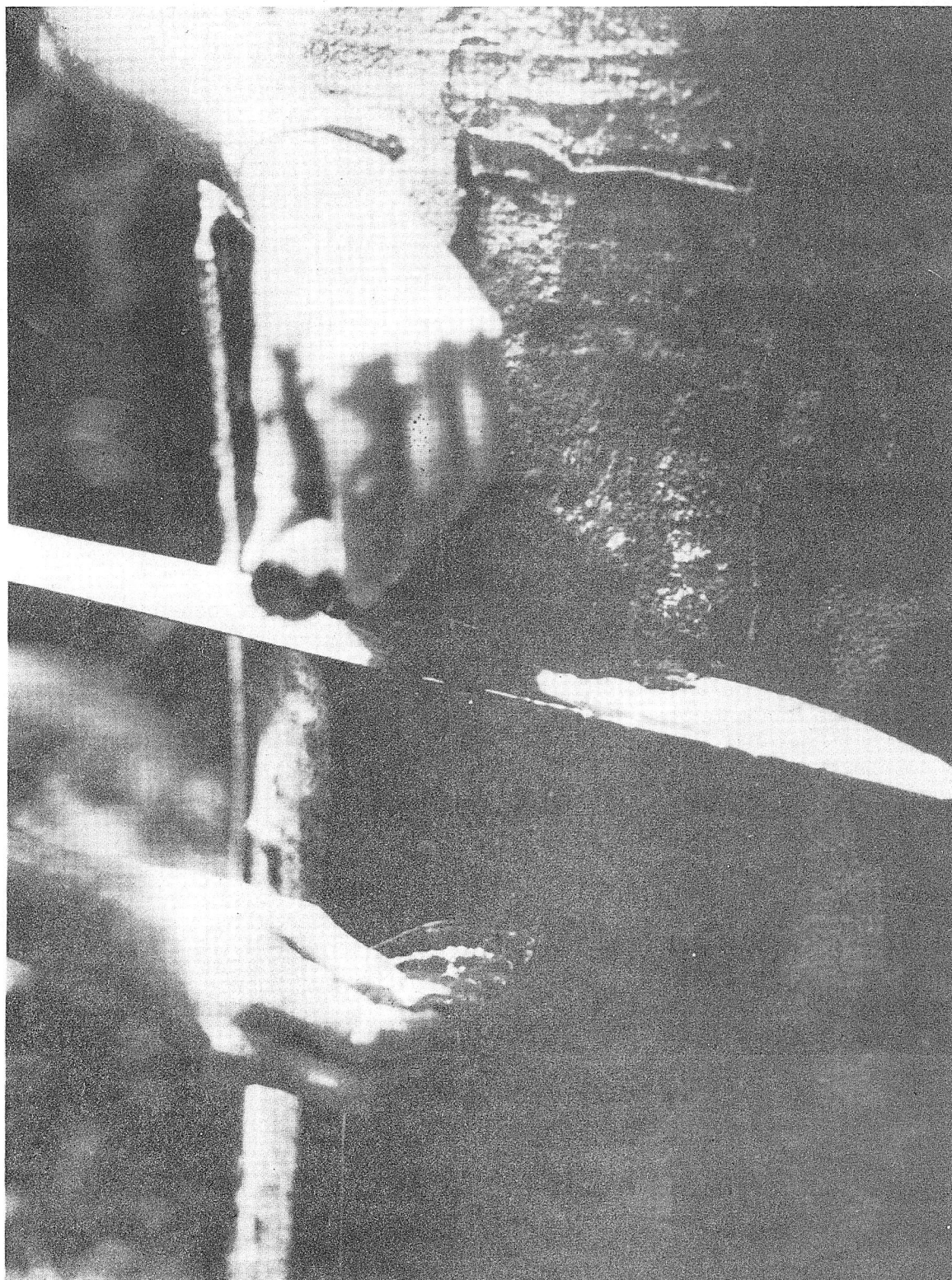


Fig. 9 Copaifera multijuga, Manaus, Brazil showing
flow of oil from bung (Ancona)

CBB 821-230

The biosynthetic method by which the diesel oil from the *Copaifera* is made is the same as that used by the *E. lathyris* up to the C₁₅ step. The *Copaifera* cyclizes the C₁₅ farnesyl pyrophosphate, that is, drops the phosphorus off to give the cyclic C₁₅ compounds. One type of enzyme is responsible for the difference in the two end products of *C. multijuga* and *E. lathyris* and that is the farnesyl pyrophosphate cyclase enzyme. With *Euphorbia lathyris* this compound is dimerized while with the *Copaifera* the material is cyclized, with many cyclic C₁₅ products as a result.

Another family, the *Pittosporaceae*, contains trees which produce fruit (nuts) which are rich in terpenes. The terpenes rather than seed oils (glycerides) seem more desirable as fuel candidates. One of the first members of the *Pittosporaceae* family which came to my attention is native to the Philippines and produces a fruit (nut) which is rich enough in terpenes to be used for illumination. This is *Pittosporum resiniferum* and the oil from the "petroleum nut" has been analyzed and found to contain a simple mixture of terpenes.^{21,22}

With the *P. resiniferum*, we can assume an average of 10% oil yield and a maximum yield of 18 kg of fruits/tree/year at a high density planting (2 meters x 2 meters). A maximum oil yield/acre/year would be 10 barrels. With smaller fruits the yield would be approximately 6.5 barrels/acre/year. The figure of 6.5 barrels/acre/year is the same as for *E. lathyris*, and the 10 barrels/acre/year is the same as the yield for palm oil which is the best oil yield so far obtained for seed crops. Therefore, the *P. resiniferum* appears to be a very desirable fuel crop if high-yielding trees can be selected and planted at high density.

We then examined the fruit of a species of *Pittosporum* which is native to California (*P. undulatum*). When the fruits were extracted they were found to have the same terpenes (C₁₀ compounds) as the ones native to the Philippines.²¹ Because this genus does grow prolifically in the Philippines and even in such places as California, these trees may turn out to be good candidates for fuel (terpene) production.

A comparison of biosynthetic routes might be of interest at this point. In the case of *Euphorbia lathyris*, the sequence is all the way to C₁₅ compounds followed by dimerization to C₃₀ materials. In the *Copaifera* (diesel tree), the sequence goes all the way to C₁₅ followed by cyclization to the sesquiterpenes. For the *Pittosporum*, however, the route is to the C₁₀ compounds, followed by cyclization to create monoterpenes in the fruit.

GENETIC ENGINEERING

The question now arises as to whether it is possible to transfer the gene for the production of sesquiterpenes from the *C. multijuga* (or other *Copaifera* species) to such plants as *Euphorbia lathyris*.^{8,11} It is not yet possible to grow *Copaifera* in the United States and, therefore, gene transfer seems to be a method of using the characteristics of sesquiterpene production in that species in another plant species, which can be grown on an annual basis under conditions which exist in the United States and elsewhere. There is only one enzyme, farnesyl pyrophosphate cyclase,

involved to move the C₁₅ pyrophosphate to a cyclic C₁₅ instead of having the compounds go all the way to C₃₀ as is the normal route in the Euphorbias. In other words, a single gene transplant from a donor cell of the *Copaifera* to the acceptor cell of a plant such as *Euphorbia lathyris* would be required. However, it is necessary to find a donor cell which has the genes for the desired enzyme, take out the messenger RNA, make a copy of the DNA, insert the copy DNA into a plasmid, clone the plasmid in *E. coli* and then, by means of the plasmids or other vector, insert the gene into a selected plant. Eventually, the piece of genetic information can be integrated into the nuclear gene of the transformed cell which has been done with bacteria but not yet accomplished with higher plants.

The regeneration of whole plants has not yet been accomplished through genetic engineering, although some plants, such as tobacco and carrot, have been regenerated from single cells. To try and accomplish whole plant regeneration by means of genetic engineering techniques it is necessary to have the two plant species available. The *Coapifera*, as it does not grow in the United States, must be cultivated in greenhouse conditions. We managed to obtain some seeds of various species of *Copaifera* (*C. langsdorfii*, *C. lucens*, *C. multijuga*, *C. officinalis*) and have tried to germinate them without too much success. We do have, however, some plant materials which hopefully will be able to provide us with the donor cells we need to place the enzymatic information into the *E. lathyris* for transformation.

However, to accomplish gene insertion into the acceptor plant it is necessary to prepare a plant tissue culture. We have been able to take the callus tissue from *E. lathyris* and separate it into protoplasts. However, the protoplasts do not reproduce very well because they tend to aggregate into new callus tissue. We have, however, separated single protoplasts from the callus and they do divide and form new callus in only a few hours. We have also regenerated whole plantlets from single protoplasts through such a callus.

One alternative method to introduce new genetic material into plant protoplasts would be to use the technique of cell fusion, that is, take a cell that has the desired gene and fuse it with a cell that does not, then select the plant fused cells.²³ Eventually there should be a cell into which the desired gene has been integrated. We have used a cell selection system and measured the process by flow cytometry. We took two sets of protoplasts from *Euphorbia lathyris*, staining one of them with rhodamine and one with fluorescein, both of which fluoresce in different regions of the spectrum. The two cell lines were mixed, a fusion agent was added so that the fused cells can be selected. This selection is done via flow cytometry, a method which passes the cells in front of a laser beam.²³ Two photomultipliers on each side of the laser beam on the flow cytometer examine light of different wavelengths. As the cells go by, a charge is placed on them only if they have both types of fluorescence. That charge allows separation of the charged from uncharged (fused from unfused) cells.

The next step in the process is to regenerate plants from leaf protoplasts which can be done (Fig. 10). Whole plants have just been regenerated in this way.²⁴ The next step will be to examine the regenerated shoots to see if any of them make cyclic C₁₅ compounds, at which point we can begin to optimize the process and its steps to produce the new plant material which will be able to synthesize the sesquiterpene compounds for fuel and materials use.

CONCLUSION

The use of plants for fuel is not a new idea. Two different efforts were made over 40 years ago in this direction. The first was an attempt by the Italians in Ethiopia to use a member of the Euphorbiaceae family, E. Abyssinica, as a source of fuels. These plants grew prolifically throughout Ethiopia and the Italians built an extraction plant for the oils from these plants. However, time did not permit the development of this idea and the Italians abandoned the effort when they left Ethiopia in 1938. They had hoped to use this plant species, which contained oil, as the basis of a petroleum industry.²⁵ In addition, the French in Morocco in 1940 used another species of Euphorbia, E. resiniferum, and succeeded in obtaining oil from wild plants. Their yield was 3 metric tons of oil/hectare from wild plants;²⁶ they did not continue this effort, however.

It is clear that the idea of using plants to create hydrocarbon-like materials as a substitute for petroleum will become more important, especially in some of the less developed areas of the world which have land unsuitable for food production. Various efforts have been made--in Spain, Okinawa, Thailand, Australia--toward this end, and attempts are underway to improve agronomic yields, develop small scale extraction plants and learn more about the composition of the oil itself, using species of plants that heretofore have not been used for large-scale cultivation. In the United States efforts along these lines in the southwest and California have shown that it would be possible to produce approximately barrels of oil/acre/year on semiarid land.

What is now needed is an effort on the part of the agricultural community to commit itself to an "energy" agriculture which would have long term benefits for our country. However, it is my feeling that we will have to be shown by others that this type of development is feasible, before we begin to use this most important resource, the annually renewable green plant, for our own benefit.

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Fig. 10 Euphorbia lathyris shoot from protoplast callus.
(Redenbaugh and Kawaguchi, Plant Genetics, Inc.)

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